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January 30, 2005

Docket 03-101-2  
Regulatory Analysis and Development, PPD, APHIS  
Station 3C71, 4700 River Road Unit 118  
Riverdale, MD 20737-1238

Dear Sir or Madam:

Please find enclosed 4 copies of a risk analysis paper, *Roundup Ready Creeping Bentgrass: The Implications of Deregulation*, which constitute my comment on Docket 03-101-2.

Ms. Judy L. Lee (APHIS) has informed me that my comment will be acknowledged and read.  
Thank you for accepting my comment late.

Sincerely,



Daniel Choi

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# **Roundup Ready Creeping Bentgrass**

## **The Implications of Deregulation**

Daniel Choi  
January 13, 2005

## BACKGROUND

Creeping bentgrass (*Agrostis stolonifera*) is a high-maintenance turfgrass used primarily in golf courses and putting greens. It can be mowed to mere fractions of an inch to attain the carpet-like texture preferred by golfers. It is a wind-pollinated perennial grass, and its small seeds (up to  $6 \times 10^6$  per pound) are easily dispersed by wind, water and animals. Outside of golf courses, creeping bentgrass is usually considered a weed; its name, "creeping," comes from its lateral invasiveness. It is sexually compatible with a number of other grass species; Watrud et al. reports natural hybrids of *A. stolonifera* with at least six other native species of the genus *Agrostis*. First-generation hybrids tend to be low in fertility or sterile, but given the right conditions some hybrids can displace both parent species. Sterile hybrids, like creeping bentgrass itself, exhibit lateral vegetative spread via stolons (9).

Maintaining the aesthetic quality and playability of bentgrass greens requires significant effort on the part of golf course caretakers. The most daunting task they face frequently is the control of weeds, especially *Poa annua*, a bluegrass species. Monsanto and Scotts' genetically modified Roundup Ready creeping bentgrass, abbreviated as RRCB (*A. stolonifera* L., event ASR368), was developed to help simplify what is still an expensive and chemical-intensive task for most golf courses. RRCB is resistant to glyphosate, the main active ingredient in Monsanto's Roundup herbicide. Glyphosate hinders the action of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a crucial enzyme in plant metabolism. RRCB can tolerate the effects of glyphosate by virtue of an EPSPS gene from *Agrobacterium* sp. strain CP4; the enzyme produced when the bentgrass expresses the bacterial gene can resist the effects of glyphosate. The EPSPS produced by RRCB is identical to that produced by other Roundup Ready crops, like soybean and corn (3).

## ANALYSIS

Roundup Ready creeping bentgrass poses a unique problem as the first perennial, highly outcrossing transgenic plant to come up for deregulation. If commercialized, it could potentially be introduced into numerous residential environments by extensive use in golf course greens and fairways. As the long-term physiological and ecological effects of genetic manipulation are as yet unknown, any decision on the status of RRCB must be made with caution. RRCB has some obvious benefits to offer to golf course caretakers, but it is not a fail-safe development.

Appendix I of USDA petition #03-104-01p submitted by Monsanto and Scotts lists several advantages of RRCB use, the most important being the potential reduction or replacement of 500,000 pounds of pesticidal active ingredient with less-toxic glyphosate, and the ability to implement a greatly simplified green and fairway management program (3). The perceived environmental benefit here is significant, but several uncertainties must be taken into account when considering these benefits, namely:

- The effects of increased glyphosate application on its surrounding environment,
- The weediness of hybrids formed between RRCB and native grass species,
- The phenotypic similarity of RRCB to unmodified creeping bentgrass.

## **GLYPHOSATE**

Glyphosate, widely used as the active ingredient in herbicides like Roundup and Rodeo, is considered to be relatively non-toxic. It is readily absorbed by soil, where microorganisms can break down glyphosate as well as common surfactants into inert substances (6). The EPA classifies glyphosate as a category E pesticide in terms of carcinogenicity, meaning that glyphosate is not likely to be a carcinogen. Lab tests on rats and rabbits indicate only a slight acute toxicity. However, as with numerous chemicals the long-term toxic effects of glyphosate on animals and humans are still unclear. Also unclear is the herbicide's potential ability to contaminate groundwater supplies or stream sediments, an important piece of knowledge necessary to determine if the introduction of RRCB would reduce herbicide runoff from golf courses (5). Commercializing RRCB inherently calls for replacing a collection of various herbicides and plant growth regulators with Roundup, which may decrease overall environmental exposure to pesticidal active ingredient but will still lead to an increase in exclusive exposure to glyphosate. Quantitative study into long-term effects of increased levels of glyphosate on animals and humans is necessary in order to predict with greater certainty that the raised glyphosate exposure that RRCB use will almost certainly bring to residential communities is ultimately safe.

Another concern frequently heard regarding extensive use of a single herbicide is the inevitable natural selection of weeds that are resistant to the herbicide, which would render the chemical useless. Golf courses with RRCB greens or fairways will almost certainly make extensive or even exclusive use of Roundup herbicide. However, resistance to glyphosate does

not readily develop in weed populations treated with the chemical; Roundup has been in use for about 30 years without many reports of major problems due to resistance. Since the EPSPS protein, which glyphosate targets, is an integral part of plant metabolism, most changes in the protein that would confer resistance to a weed end up being fatal to the weed (7). Therefore, increased use of glyphosate following the introduction of RRCB to golf courses is unlikely to lead to the development of glyphosate-resistant plant pests, at least for a few decades. Caretakers must still exercise discretion in their use of Roundup, since several weeds have been reported to be glyphosate-resistant. Without a sound application program designed to slow the selection for resistances, extensive glyphosate use would pose more of a risk than it should.

## **HYBRID WEEDS**

RRCB is wind-pollinated, which raises concerns of its hybridizing with sexually compatible grass species outside of a golf course. Creeping bentgrass has been reported to form hybrids with at least 13 grass species of *Agrostis* and *Polypogon* in the US (1). Hybrids formed between RRCB and any of these species would probably inherit glyphosate tolerance. Since bentgrasses are usually considered to be weeds in the residential environment, the introduction of glyphosate tolerance to these grasses could pose problems for homeowners. According to the EPA, glyphosate was the second most frequently used herbicide in the home and garden sector in 2001, with 5-8 million pounds applied. The only herbicide used more often was the toxic substance 2,4-D, and the number does not include professional applications of glyphosate to home lawns and gardens. Commercialization of RRCB may lead to the establishment of glyphosate-resistant hybrid weeds in residential areas that would be able to resist the effects of one of the herbicides most commonly used by homeowners.

Most of the hybrids that RRCB forms with other species are either sterile or low in fertility. However, this characteristic does not preclude some of the hybrids from being vigorous, and a few competitive hybrids would be able to establish themselves by vegetative propagation. The weediness of these hybrids depends to an extent on environmental conditions and the aggressiveness of other established species.

The light seeds of RRCB also pose a contamination problem, since they can readily be introduced into lawns and grass seed used by homeowners (9). The RRCB itself would then pose a weed risk that again could not be controlled using glyphosate.

Monsanto and Scotts propose mechanical methods and the alternative herbicides fluazifop, sethoxydim and clethodim as means to control any glyphosate-resistant weeds that form due to RRCB stands (3). Using the alternative herbicides suggested, all of which are ACCase inhibitors, in fact is more likely to promote selection of resistant weeds than using glyphosate (8). If glyphosate-resistant weeds from RRCB outcrossing and contamination do succeed in establishing themselves in residential areas around golf courses, excessive use of “high-risk” herbicides like fluazifop may encourage the development of additional herbicide resistances. In addition, if using the alternative herbicides became a necessity, such usage would at least partially offset the decreases in herbicide used in golf courses.

Professor Zac Reicher of Purdue University, in a supplementary letter provided with the petition, suggests studying atrazine as another alternative to glyphosate (3). Atrazine would not be particularly useful substitute in the home environment, since it is classified as a known carcinogen and groundwater contaminant (4).

Creeping bentgrass could readily transfer any herbicide resistance it develops naturally to any of its relatives, and CP4 EPSPS-induced glyphosate resistance would not necessarily spread more or less readily than natural resistances. In the absence of glyphosate, the resistance trait probably would not confer a competitive advantage to RRCB and its hybrids (3). Therefore, there is little reason to believe that the CP4 EPSPS gene would spread rampantly through bentgrass populations. However, the effect of introducing the CP4 gene into residential environments cannot be shown with any certainty to be completely benign. “Superweeds” may not immediately form due to contaminants from RRCB, but the transfer of the CP4 gene into areas outside the controlled environment of a golf course could still cause problems that range anywhere from minor to significant.

## **EQUIVALENCE OF PHENOTYPES**

Numerous chemical and field tests of RRCB conducted between 1999 and 2002 in various locations, focusing on a variety of properties chosen to determine potential weediness, led Monsanto and Scotts to conclude that RRCB is essentially the same as wild-type creeping bentgrass save for the resistance to glyphosate. However, the full effects of the introduction of a foreign gene into a host organism are not well understood. It is important to note that the CP4 EPSPS produced by RRCB, though homologous to plant EPSP synthases, is not the same protein.

Small differences, especially in a crucial metabolic protein like EPSPS, can have significant effects on the host organism. The transgenic EPSPS may not function as efficiently or specifically as the natural EPSPS, or it may interact with other substrates or respiratory components of the organism, producing effects that bentgrass may not naturally experience. In most cases, such effects would decrease the fitness of the grass, rendering it more susceptible to stresses. The production, use, and presence of CP4 EPSPS in RRCB may make the grass more vulnerable to diseases and fungal or insect pests, which would lessen the benefits of a decrease in overall herbicide use by requiring increased insecticide or fungicide usage. In addition, RRCB may end up being less heat or traffic tolerant than the natural type, and may have longer recovery times following frequent mowing. In that instance, it may not be very cost-effective to replace natural bentgrass in golf courses with RRCB. These questions of fitness are not easily answered by short-term field tests or even by chemical analyses, since problems often become apparent only after the transgenic plant has been acquiesced to the conditions and competitive pressures in environment of major use.

Reports of stem splitting in Roundup Ready soybean have raised questions about the effects of transgene introduction on the host plant (New Scientist, 20 Nov 1999). This issue has arisen after several years of deregulated use. While it cannot be traced with certainty to the genetic modification, it does indicate that careful long-term study of transgenic plants must be conducted to accurately determine their equivalence to their conventional sources.

## **RECOMMENDATIONS**

Monsanto and Scotts' comprehensive study of RRCB revealed little evidence of a plant pest risk, at least with short-term use. While the commercialization of RRCB probably will not have immediate and dire consequences like the rapid establishment of glyphosate-resistant superweeds, our general lack of knowledge on the environmental effects and implications of introducing genetically modified organisms to the market should warn federal authorities to deregulate such organisms with extreme caution. The USDA should not deregulate RRCB until the uncertainties previously described, all surrounding the long-term use of RRCB, can be resolved.

The USDA should not fully approve the petition for deregulation of RRCB, especially since RRCB has very limited uses. Its benefits are tangible only to golf course caretakers,

exacting golfers, and golf enthusiasts, a group that constitutes just a small portion of the American population. Arguments of reduced pesticide usage in golf courses are difficult to quantify as of now, and given the increase in pesticide use on fields of Roundup Ready corn (2), they may never be validated. Reductions in the cost of maintaining golf courses may be partially or even fully mitigated by the effects of introducing and extensively using hazardous pesticides like atrazine or fluazifop on home lawns. In the course of settling this matter the USDA must be sure to compare the number of people likely to fully accept the uncertainties and risks involved with using RRCB (26-37 million golfers and enthusiasts in the US) to the number of people who would object should any of the proposed risks turn out to be a real problem (hundreds of millions of homeowners living in suburbs, a good portion near golf courses and other controlled turf environments, in addition to the managers of any forest or preserve areas nearby). It cannot be definitively proven that RRCB will not bring negative effects with it to residential environments, and given that a majority of people in residential areas would not enjoy living with Roundup Ready weeds or finding out that large doses of glyphosate pose a health risk, the USDA should be careful not to give Monsanto and Scotts the stamp of approval without first subjecting RRCB to more rigorous and revealing tests.

Although immediate approval of the petition should be a near impossibility, more information on the long term effects of RRCB would be helpful in determining whether the grass, and ultimately other genetically modified organisms, can be used toward beneficial purposes without causing problems or annoyances in surrounding areas, or even in the area of use. The uncertainties previously discussed all could potentially diminish the benefits of RRCB, and currently there is not enough information to dispel any of them. Information beyond that can be obtained in controlled field tests is necessary in order to address such issues.

Extended study for additional information would be possible if the USDA instates a 6-10 year period to evaluate the utility and the problems of RRCB. Monsanto and Scotts should not be allowed to widely market the product during this period, instead supplying the product to a few selected golf courses in different parts US. Courses should be selected based on climate (preferably hotter climates, especially in Southern regions where native bentgrass and bentgrass relative populations tend to be low and where extensive field testing of RRCB has not yet occurred), ample distance from national park or preserve areas, and any other factors the USDA deems necessary to protect public interests. Grass quality and genetic composition, as well as



pesticide usage, should be carefully monitored by a combination of course caretakers, Monsanto and Scotts, and the USDA. Monitoring weeds around the site during the trial period would also prove to be useful, as would thorough study of the potential health risks of glyphosate. RRCB seed would be produced only for limited use in test sites, in carefully isolated environments as described by Monsanto and Scotts in their petition (section VII E), and grass seeds could be screened for the presence of RRCB seeds.

This trial period would provide the data that would allow expert scientists to more accurately assess the long-term impacts of the commercialization of RRCB. Critical questions like the transgenic grass' equivalence to its non-transgenic source could be answered with greater certainty. Analyses conducted of the areas surrounding the golf course during the trial period may help elucidate the impact of RRCB on the residential environment in which it is likely to be used. Whereas the information presented in the petition, coming from no more than a year or two of study, elucidate only the short-term safeness of RRCB, 6-10 years of study in the proposed environment of future use will probably provide at least a glimpse of the long-term effects of RRCB use.

It is especially vital to proceed with caution on the decision regarding RRCB since this decision will be viewed as a precedent in the future. RRCB, if commercialized, will be the first perennial, wind-pollinated, highly outcrossing transgenic plant to enter the market and, unlike Roundup Ready crop plants, to be used on a moderate scale close to major residential areas. Its deregulation could be used as a stepping-stone by other companies to secure the deregulation of other such transgenic plants with specific features for specific uses in any aspect of American life. The USDA should therefore conduct extensive study on RRCB to gain a more concrete idea of the risks and benefits for transgenic organisms, which would help in determining pertinent issues to be considered before deregulating transgenic organisms in the future. The 6-10 year "trial period," applied in the current case regarding RRCB, may lead to findings that could help guide decisions on transgenic organisms in the future, or it can be consistently applied to different transgenic organisms that come up for deregulation until the implications of such decisions are known with greater certainty.

## REFERENCES

1. APHIS Preliminary Risk Assessment on the Petition for a Determination of Nonregulated Status for Creeping Bentgrass (*Agrostis stolonifera*) Genetically Engineered (Event ASR368) for Tolerance to the Herbicide Glyphosate submitted by Monsanto Company and the Scotts Company. Dec 2003. <[http://www.aphis.usda.gov/brs/aphisdocs/03\\_10401p\\_ra.pdf](http://www.aphis.usda.gov/brs/aphisdocs/03_10401p_ra.pdf)>.
2. Benbrook, Charles M. "Factors Shaping Trends in Corn Herbicide Use." Ag BioTech InfoNet, Northwest Science and Environmental Policy Center. 23 Jul. 2001. <[http://www.biotech-info.net/corn\\_reduct.html](http://www.biotech-info.net/corn_reduct.html)>.
3. Frelich, Jim, Scott Huber, Eric Nelson, and Terry Stone. Petition for Determination of Nonregulated Status: Roundup Ready® Creeping Bentgrass (*Agrostis stolonifera* L.) Event ASR368. Apr 2003. <[http://www.aphis.usda.gov/brs/aphisdocs/03\\_10401p.pdf](http://www.aphis.usda.gov/brs/aphisdocs/03_10401p.pdf)>.
4. "Atrazine - Identification, toxicity, use, water pollution potential, ecological toxicity and regulatory information." Pesticide Action Network (PAN) Pesticides Database. 2004. <<http://www.pesticideinfo.org>>.
5. Ibid, "Glyphosate - Identification, toxicity, use, water pollution potential, ecological toxicity and regulatory information."
6. Information Ventures, Inc. Glyphosate: Pesticide Fact Sheet. Nov. 1995. <<http://infoventures.com/e-hlth/pesticide/glyphos.html>>.
7. Jasieniuk, Marie. "Constraints on the Evolution of Glyphosate Resistance in Weeds." Resistant Pest Management. Winter 1995. <<http://www.msstate.edu/Entomology/v7n2/art16.html>>.
8. Proost, Richard and Jerry Doll. "Avoiding Herbicide Resistance in Weeds." Nutrient and Pest Management, University of Wisconsin-Madison. Nov 2002. <<http://ipcm.wisc.edu/pubs/pdf/AvoidHerbResis2002A3615.pdf>>.
9. Watrud, Lidia S. et al. "Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with *CP4 EPSPS* as a marker." Proceedings of the National Academy of Sciences. 101 (2004): 14533-14538.